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Research Article

Cardiovascular Risk Assessment of Adult Male Wistar Rats Acutely Exposed to Waterpipe Smoke Emitters

Baiyekusi Samuel[1], Akor-Dewu Maryam B[1], Tende Joshua A[1], Ibrahim Suleiman[1], Dubo Augustine B[1]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria.

[*]***Author for Correspondence:***
Baiyekusi Samuel
E-mail: sam2.baiyekusi@gmail.com

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ABSTRACT

Introduction: Alarming statistics on cardiovascular-related causes of death have signalled the need for studies on risk factors leading to cardiovascular disease. Waterpipe smoking, being a form of tobacco use has evidently taken the centre stage in recent times, as the habit of choice for pastime among youths of both genders.

Objective: This study is therefore aimed at assessing the effect of waterpipe smoke emitters on cardiovascular risk generation.

Methods: Twenty (20) adult male Wistar rats were employed for the study and randomly grouped into four (five per group). They were exposed to ambient air (control), charcoal smoke (Group 2), tobacco smoke (Group 3) and flavoured tobacco smoke (Group 4) respectively for 10 days through a nose-only exposure system. Concentrations of serum high-sensitivity C-reactive protein and cardiac homogenate inducible nitric oxide synthase and tumour necrosis factor-alpha were assessed for cardiovascular risk using ELISA methods.

Results: There was no significant difference in concentration of high-sensitivity C-reactive protein ($p>0.05$) in all smoke exposed groups when compared to control group. However, there was statistically significant increase in concentration of inducible nitric oxide synthase ($p<0.05$) in tobacco exposed group (18.43 ± 0.16) when compared to control group (17.31 ± 0.18). Also, there was a statistically significant increase in concentration of tumour necrosis factor-alpha ($p<0.05$) in flavoured tobacco exposed group (590.98 ± 9.76) when compared to control group (554.68 ± 7.96).

Conclusion: Outcomes of this study suggests that waterpipe smoke emitters may only cause cardiovascular risk in users when taken in combination.

INTRODUCTION

Tobacco smoking is a major preventable cause of morbidity and mortality in low and middle-income countries.[1,2] It is known to be responsible for about 6 million deaths per year worldwide, which makes it the second major leading cause of death and is currently responsible for one in ten deaths among adults across the world.[2] Waterpipe, also known as Shisha, hookah, arghile, narghile and hubble bubble, has become a conventional way of smoking tobacco in recent times amongst young people of both genders.[3] Although it is a practice that dates back at least, four centuries, its increasing popularity has been linked with the widely accepted incorrect perception of it being a less harmful form of tobacco use.[4,5]

Its origin has been traced to Eastern Mediterranean region, which comprises countries of the Middle East and North African region and has spread across western countries such as Australia, United Kingdom, Canada and the United States.[6,8] In Nigeria, it is popularly seen in club houses and Shisha cafes where people use it as a pastime. Many published articles on the prevalence of waterpipe smoking have focused on Asia, Europe, North Africa, North and South America, but there are only a handful of published statistics on its prevalence around sub-Saharan Africa where Nigeria is located.[9]

Cardiovascular disease remains the leading cause of death throughout the world.[1,2] WHO,[2] in annual report on cardiovascular diseases revealed that approximately 17.9 million (31%) of all deaths worldwide in 2016 were caused by cardiovascular disease, and 37% of this number were from cardiovascular disease related deaths occurring in low-income and middle-income countries. The role of inflammation in atherosclerosis and its acute clinical manifestations in heart attacks and strokes has been backed by significant association with very high concentrations of serum interleukin-6 (IL-6) – a hallmark of inflammation, which is one of the most potent drivers of C-reactive protein (CRP) production in hepatocytes.[10,11] In recent times, high-sensitivity C-reactive protein (hs-CRP) has received the most attention as the biomarker of choice for screening and risk reclassification in cardiovascular disease states, perhaps, because it is a highly sensitive assay of CRP which can

accurately detect very low levels of CRP in apparently healthy individuals.[12] Inflammation is implicated in all stages of atherothrombosis, which is the underlying cause of sudden cardiac death.[13,14]

Although preventive measures such as the use of aluminium foil and water base as filter to reduce the amount of toxicants inhaled have been deployed in the use of waterpipes, there still appears to be enormous amount of toxicants in the inhaled smoke.[15,16] Very importantly, the enormous free radicals contained in the smoke cause systemic inflammation leading to baseline elevations of C-reactive protein – a serum inflammatory biomarker that increases the future risk for cardiovascular disease on exposure to free radicals, infection, trauma or stress.[17] On the other hand, nitric oxide has been reported to play an important role in inflammatory process when produced in excess by inducible nitric oxide synthase (iNOS) – an isoform of nitric oxide synthase released by the endothelium in response to inflammatory signals such as increased free radicals and cytokines.[18] Therefore, the aim of this study is to compare the cardiovascular risk of adult male Wistar rats acutely exposed to waterpipe smoke emitters by measuring the serum concentrations of hs-CRP, level of insult to the endothelial monolayer by measuring cardiac homogenate concentrations of iNOS and level of inflammation in the tissue by measuring concentrations of tumour necrosis factor alpha (TNF-α).

MATERIALS AND METHODS

Experimental Animals

A total of twenty adult male Wistar rats (Rattus norvegicus) weighing between 150-170g were purchased from the animal house of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and transported to the animal house of Department of Human Physiology, Ahmadu Bello University, Zaria. The rats were allowed to acclimatize to their new environment for a period of two weeks before the commencement of the experiment. During the study period, animals were fed on grower pelletized feed and water ad libitum and exposed to 12-hours light-dark cycle. Experiment was conducted in accordance with the Animal Use and Care Policy of Ahmadu Bello University, Zaria. The twenty adult male albino Wistar rats were grouped in four, each group having five adult male Wistar rats. The groupings are as follows:

Group 1 – Ambient air (control)
Group 2 – Charcoal smoke
Group 3 – Tobacco smoke
Group 4 – Flavoured tobacco smoke

Experimental Design

After 2 weeks of acclimatization, the control group was exposed to ambient air and the other three experimental groups were exposed to the smoke emitters. Animals were carefully placed in a soft restrain chamber and connected to an exposure tower through their noses using a nose-only exposure system.[19,20] Although each group was exposed to different constituent, the same procedure was deployed for all experimental groups. A standard of one puff for 7 seconds was taken once a minute, with 53 seconds of fresh air at a constant rate, a slight modification of Nemmar *et al*.[21] using a pressure gauge of 40mmHg. Daily sessions lasted for 30 minutes per group for 10 days. For each session, 10g of tobacco and flavoured tobacco both containing 0.5% nicotine were used with one briquette at a time.

Blood Sample Collection

Following the 10th day of exposure, animals were kept for 24 hours before been anaesthetized with Sodium Pentobarbital (60mg/kg body weight) administered intraperitonially.[21] Thorax of each Wistar rat was exposed and blood sample was collected via cardiac puncture in plain tubes and centrifuged at 1500 x g for 10 minutes. Serum was collected and assayed for hs-CRP concentration using ELISA kit purchased from Wuhan Fine Biotech Co., Ltd (China).

Cardiac Tissue Homogenization

After collection of blood via cardiac puncture, heart tissue with the greater part of aorta was quickly excised and washed in ice-cold phosphate buffer solution (pH 7.4). The tissue was minced to small pieces and homogenized in 50mM TRIS buffer containing 400mM NaCl and 0.7% Triton X-100 (pH 7.4). Homogenate was centrifuged at 1500 x g for 10 minutes, after which iNOS and TNF-α concentrations were determined using ELISA kits purchased from Wuhan Fine Biotech Co., Ltd (China).

Statistical Analysis

All data collected were expressed as Mean ± Standard Error of Mean. They were analyzed using the One-way ANOVA (Analysis of Variance) alongside Tukey's Post-hoc test. AP value less than 0.05 ($p<0.05$) was considered statistically significant. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 20.0.

Ethical Approval

Ethical approval was sought from the Ahmadu Bello University Committee on Animal Use and Care. Clearance on animal use act was given before the commencement of the study.

RESULTS

Figure 1 shows result of tissue inflammation where only flavoured tobacco exposed group (590.98 ± 9.76 ng/ml) was significantly increased ($p<0.05$) compared to control group (554.68 ± 7.96 ng/ml). The result in Figure 2 shows that there was statistically significant increase ($p<0.05$) in iNOS concentration in tobacco exposed group (18.43 ± 0.29 ng/ml) when compared to the control (17.31 ± 0.18 ng/ml). Figure 3 shows serum concentrations of hs-CRP with no statistical significance ($p>0.05$) between control and all experimental groups.

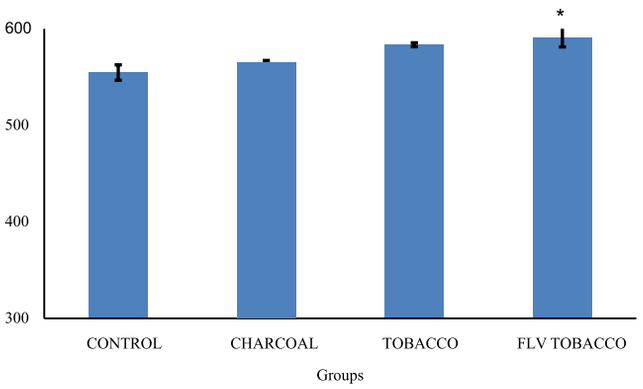

Figure 1: Mean Concentrations of Tumour Necrosis Factor-Alpha (TNF-α) in Cardiovascular Tissue Homogenates of Adult Male Wistar Rats Acutely Exposed to Waterpipe Smoke Emitters.
O.P. = Significant difference at $p<0.05$ compared to the control. FLV = Flavoured.

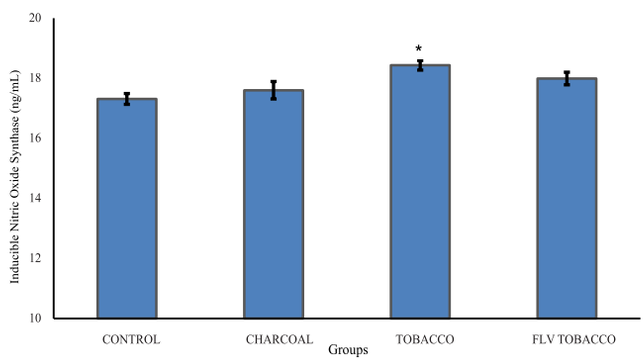

Figure 2: Mean Concentrations of Inducible Nitric Oxide Synthase (iNOS) in Cardiovascular Tissue Homogenates of Adult Male Wistar Rats Acutely Exposed to Waterpipe Smoke Emitters.
* = Significant difference at $p<0.05$ compared to the control. FLV = Flavoured.

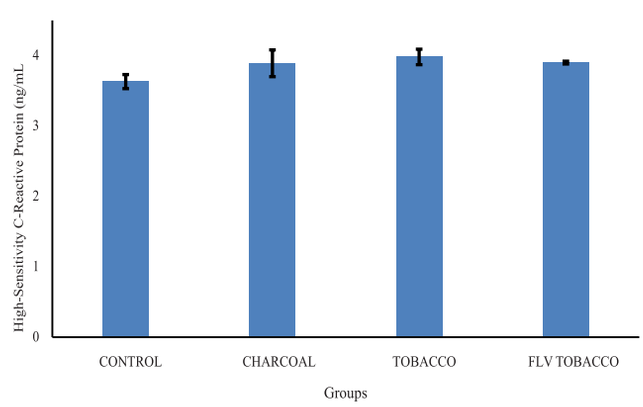

Figure 3: Mean Concentrations of Serum High-Sensitivity C–Reactive Protein (hs-CRP) of Adult Male Wistar Rats Acutely Exposed to Waterpipe Smoke Emitters. FLV = Flavoured.

DISCUSSION

In this study, we sought the effect of each emitter of smoke in a waterpipe set-up, which to the best our knowledge has not been investigated in any previous study. While numerous studies have shown that cigarette smoking is a major independent risk factor for cardiovascular disease, studies on cardiovascular risk assessment following acute waterpipe smoking remain scarce. This current study therefore attempted to determine the effect of different waterpipe smoke emitters (charcoal, tobacco and flavoured tobacco) on cardiovascular risk biomarkers.

Outcomes of this present study supports the increasing evidence that waterpipe smoking, like cigarette smoking, causes cardiovascular disease, as it reveals short-term onset of inflammation in cardiovascular tissue of adult male Wistar rats. This agrees with the findings of Nemmar *et al*.,[21] whose study reported cardiovascular inflammation in a 5-days nose-only waterpipe smoke exposure in mice. Flavoured tobacco exposed group showed statistically significant increase in tissue TNF-α concentration, which correlates with the result of Nemmar *et al*.[21] where tissue IL-6 and TNF-α were significantly increased. This outcome could be associated with increased exposure to free radicals which flavouring and humectant add to the mainstream smoke.[22] Schubert *et al*.[23] had earlier reported the role of humectants in the formation and release of carbonyl compounds, such as formaldehyde, acetaldehyde and acrolein. Under certain conditions of pyrolysis, glycerol (a prominent humectant in flavoured tobacco) is considered a precursor of acrolein.[24] Moreover, sustained acrolein exposure has been implicated for oxidant stress and inflammation in the heart.[25,26] Hence, with respect to other groups, flavoured tobacco exposed group was perhaps, affected by the added oxidants that flavouring and humectant contribute to the mainstream smoke.

On the other hand, there was evidence of cardiovascular risk in tobacco exposed group, where there was a statistically significant increase in iNOS concentration. This group was exposed to raw tobacco heated with briquette as seen in a conventional waterpipe set-up. The rationale behind this pattern was to isolate tobacco from flavouring and humectant, so as to determine the level of effect its specific oxidants (tobacco-specific nitrosamines) contributes to the generation of cardiovascular risk. The increase in iNOS concentration could be linked to the direct impact of free radicals down-regulating endogenous antioxidants in vascular endothelial cells,[25,27] or the presence of proinflammatory cytokines or exposure to infection.[28] Excessive production of iNOS drastically elevates nitric oxide levels, which destroys mitochondrial function and other cellular activities by the formation of reactive nitrogen species.[29]

Furthermore, there was no statistically significant difference in concentration of hs-CRP across all groups in this present study, which could be linked to delay in early establishment of systemic inflammation by proinflammatory cytokines in plasma.[11,12] Although there was evidence of inflammation in the tissue, early proinflammatory cytokines (IL-1β, TNF-α and IL-6)may not have peaked in plasma as reported in the study of Nemmar *et al*.,[21] where no evidence of systemic inflammation was found after assaying serum CRP, IL-6 and TNF-α.This led to the choice of a higher

sensitivity test (high sensitivity CRP) in this present study.However, duration of the study could have been too short for CRP production in this study.

CONCLUSION

In conclusion, outcomes of this study suggest that waterpipe smoking is cardio-toxic as a result of combined effect of various toxicants emanating from the different smoke emitters used in the set-up. At the time point of investigation, charcoal did not show any potential of causing cardiovascular risk, whereas, tobacco showed potential to cause endothelial dysfunction when heated with charcoal and flavoured tobacco heated with charcoal caused tissue inflammation. Thus, the conventional pattern of waterpipe smoking involving charcoal, tobacco and flavours may be considered a threat to the cardiovascular system.

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Disclosure Statement

The authors disclose no conflict of interest.

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